ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1982, p. 85-92
0066-4804/82/010085-08$02.00/0

Vol. 21, No. 1

# Azthreonam (SQ 26,776), a Synthetic Monobactam Specifically Active Against Aerobic Gram-Negative Bacteria

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Received 10 August 1981/Accepted 29 September 1981

Azthreonam (SQ 26,776) is a synthetic monocyclic β-lactam antimicrobial agent belonging to the monobactam family (Sykes et al., Nature [London] **291**:489–491, 1981), members of which are characterized by having the 2-oxoazetidine-1-sulfonic acid moiety. Azthreonam exhibits a high degree of stability to β-lactamases and is specifically active against aerobic gram-negative bacteria, including *Pseudomonas aeruginosa*. Its activity against these organisms was in general equal or superior to that observed with the third-generation cephalosporins, cefotaxime and ceftazidime. Like penicillins and cephalosporins, azthreonam interacts with essential penicillin-binding proteins of gram-negative bacteria. Azthreonam protected mice against experimental infections produced by a range of gram-negative bacteria, exhibiting efficacy comparable to that of cefotaxime and ceftazidime.

The discovery of monocyclic β-lactam antibiotics produced by bacteria (4, 13) has once again highlighted the enormous potential of microorganisms in producing unique and medicinally important molecules. The history of classical β-lactam antibiotics has demonstrated that alteration of the amido side chain in addition to molecular substitution at other points of the molecule can lead to dramatic changes in bioactivity, β-lactamase stability, and pharmacokinetic properties. Structure-activity relationships among the monobactams (C. M. Cimarusti, H. Breuer, T. Denzel, D. K. Kronenthal, U. D. Treuner, D. P. Bonner, and R. B. Sykes, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 21st, Chicago, abstr. no. 487, 1981.) have led to the development of azthreonam [2Sα,3β(Z)]]-3-[[(2-amino-4-thiazolyl)-[(1-carboxy-1-methylethoxy)imino]-2-methyl-4-oxo-1-azetidinesulfonic acid) (Fig. 1). This report summarizes the in vitro and in vivo properties of this novel synthetic antimicrobial agent.

## MATERIALS AND METHODS

**Antibiotics.** Azthreonam was prepared in our own laboratories and used as the disodium salt in all experiments. Cephaloridine was obtained from Eli Lilly & Co., Indianapolis, Ind.; cefoperazone was from Pfizer Inc., New York, N.Y.; cefotaxime was from Hoechst-Roussel; ceftazidime was from Glaxo; cefsulodin was from Abbott Laboratories, North Chicago, Ill.; and cefoxitin was from Merck & Co., Inc., Rahway, N.J.

**Organisms.** The bacterial isolates used were clinical isolates, identified to species by the API system (Analytab Products, Plainview, N.Y.). All isolates were stored in sealed vials in liquid nitrogen. These stock suspensions were used to inoculate plates of brain heart infusion agar which were then incubated for 18 h at 37°C. Mueller-Hinton broth (10 ml) was inoculated from these plates to provide log-phase cultures used for minimal inhibitory concentration (MIC) tests.

**MIC determinations.** Antimicrobial activity was determined by an agar dilution method. For nonfastidious organisms the medium employed was antibiotic assay broth (Difco Laboratories, Detroit, Mich.) supplemented with 1.5% agar. The susceptibility of *Bacteroides* was tested on Mueller-Hinton agar supplemented with 5% lysed sheep blood and 0.5% vitamin K; the susceptibility of streptococci was tested on Mueller-Hinton agar supplemented with 5% sheep blood; and the susceptibility of *Haemophilus* and *Neisseria* was tested on chocolate agar. A final inoculum of $5 \times 10^5$ colony-forming units (CFU), prepared by dilution of a fresh overnight broth culture, was applied to agar by a multipoint inoculator (Denley Instruments Ltd., Bolney, Sussex, England). For studies involving inoculum size, final inocula of $10^6$, $10^4$, and $10^2$ CFU were prepared and inoculated as above. Plates were incubated at 35°C for 18 h. For *Bacteroides*, incubation was carried out in Gas-Pak jars (BBL Microbiology Systems, Cockeysville, Md.),

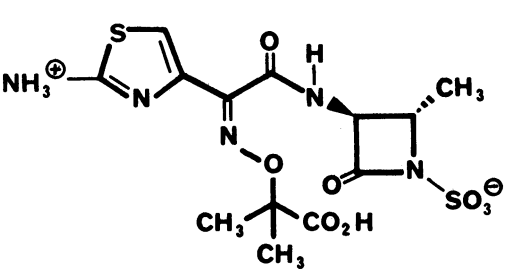

FIG. 1. Structural formula of azthreonam.

and for streptococci, *Haemophilus*, and *Neisseria* incubation was carried out in an atmosphere of 5% $CO_2$. The MIC was defined as the lowest concentration of antibiotic that inhibited development of visible growth on agar. At higher inoculum levels, where faint hazes often appeared at the site of inoculation, the plates were flooded with 2% tetrazolium chloride [2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl-2H-tetrazolium chloride] (Eastman Chemicals, Rochester, N.Y.) to distinguish haze from growth.

**Preparation of β-lactamases.** TEM-1 β-lactamase was purified from *Escherichia coli* by the method of Melling and Scott (8). TEM-2 β-lactamase from *E. coli* W3310 was purified as follows. Cells were disrupted by a freeze-thaw technique in which cells at a concentration of 1.0 g/ml were suspended in 0.2 M sodium acetate buffer (pH 5.0), frozen in a dry ice-acetone bath, and subsequently thawed at 60°C (three times). After centrifugation and dialysis, the supernatant was purified by DE-52 and Sephadex G-75 chromatography (8). Purification to homogeneity was accomplished on DE-52 by using gradient elution at pH 7.0 with the phosphate buffer concentration ranging from 8 to 16 mM. P99 β-lactamase from *Enterobacter cloacae* SC 10,435 was a homogeneous preparation purified as described by Ross and Boulton (11). The *Pseudomonas* specific enzymes (PSE 1-4) were purified from *Pseudomonas aeruginosa* strains by using the freeze-thaw procedure to break the cells, followed by elution from Sephadex G-75 (5.0 by 53 cm) in 50 mM phosphate buffer (pH 7.0). K-1 from *Klebsiella pneumoniae* SC 10,436, SHV-1 from *Klebsiella pneumoniae* SC 10,999 and OXA-2 from *E. coli* SC 10,730 were purified as follows. Cell suspensions (1:10 [wt/vol in 0.1 M phosphate buffer, pH 7.0) were sonicated for 2 min and centrifuged; the resulting supernatant was passed through Sephadex G-75. β-Lactamase activity was concentrated in a 95% ammonium sulfate fraction followed by desalting on PD-10 Sephadex. β-Lactamase preparations from *Serratia marcescens* SC 12,505 (S1), *Proteus vulgaris* SC 10,986 (P1), *Enterobacter aerogenes* SC 12,535 (E1), *Providencia stuartii* SC 10,188 (PD1), and *Bacteroides fragilis* SC 12,664 (B1) were used as supernatants of crude sonicates without further purification. β-Lactamases were characterized by isoelectric focusing (LKB Multiphor; pH range 3.0 to 9.5); enzyme activity was detected on the gels by using a chromogenic cephalosporin substrate (7). Plasmid-mediated β-lactamases were also identified through substrate profiles (6).

**Hydrolysis studies.** β-Lactamase hydrolysis studies were performed spectrophotometrically on a Gilford 250 spectrophotometer. UV spectra were recorded for each compound in the presence and absence of a selected β-lactamase. On the basis of the resulting difference spectrum an appropriate wavelength was selected for assay, and a molar extinction coefficient ($\Delta\varepsilon$) was calculated from the differential absorbance change at that wavelength (12). For analysis of high concentrations of substrate in kinetic studies, the wavelength selected for cephaloridine and aztreonam did not correspond to the wavelengths associated with the maximum absorbance change in the difference spectra.

Spectral parameters used in these studies were: cephaloridine, 295 nm, $\Delta\varepsilon$ = 890; cefoperazone, 275 nm, $\Delta\varepsilon$ = 8,460; cefotaxime, 267 nm, $\Delta\varepsilon$ = 6,700; ceftizoxime, 258 nm, $\Delta\varepsilon$ = 4,060; ceftazidime, 260 nm, $\Delta\varepsilon$ = 8,660; cefsulodin, 262 nm, $\Delta\varepsilon$ = 10,200; azthreonam, 318 nm, $\Delta\varepsilon$ = 620. All compounds were prepared in 0.1 M phosphate buffer (pH 7.0) immediately before use. Kinetic studies were performed at 25°C. A range of substrate concentrations was selected for each compound by estimating $K_m$ values from direct linear plots of raw data (2). Each determination of kinetic parameters included at least five concentrations of substrate spanning the $K_m$ value where possible. Linear regression analysis of Lineweaver-Burk plots were used to obtain the reported kinetic values. Results were calculated as described below, using the value "efficiency of hydrolysis" to describe a comparable parameter defined by Pollock (9) as "physiological efficiency:" efficiency of hydrolysis = ($V_{max}$/μl of enzyme)/$K_m$. This value takes into account not only the maximal rate for substrate hydrolysis, but also the affinity with which substrate binds to enzyme. All values obtained were then normalized with respect to cephaloridine. In cases where $K_m$ could not be determined (no detectable hydrolysis), a maximal value for $V_{max}$ was established, and a "relative $V_{max}$" value was reported: relative $V_{max}$ = [($V_{max}$/μl of enzyme) of substrate]/[($V_{max}$/μl of enzyme) of cephaloridine] × 100. For most compounds "relative efficiency" values were calculated: relative efficiency = (efficiency of hydrolysis of substrate/efficiency of hydrolysis of cephaloridine) × 100.

**Penicillin-binding proteins.** Organisms were grown overnight in brain heart infusion broth at 37°C and sonicated, and the membranes were solubilized in 2% Triton X-100 as previously described (3). Approximately 100 μg of solubilized membranes was incubated with the appropriate amount of azthreonam (final concentration, 0, 0.1, 0.5, 2.0, 10, or 100 μg/ml) at 30°C for 10 min in a total volume of 50 μl. [$^{14}$C]penicillin G (10 nmol) was added, and incubation was continued for an additional 10 min. Protein was precipitated with acetone and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (5) followed by fluorography (1). Protein-bound penicillin was determined by visual examination of the developed X-ray film. Typical time of film exposure was 20 days.

**Experimental chemotherapy.** Protection tests were done in Charles River CF-1 female mice (16 to 18 g). Mice were challenged by the intraperitoneal route with the test organisms suspended in 1.0 ml of 7% hog gastric mucin (American Laboratories, Inc., Omaha, Neb.). Organisms used in the protection tests were maintained frozen at −70°C. Ampoules containing the organisms were thawed at 37°C for 30 min and diluted to the required infectious inoculum. The challenge suspensions contained at least 100 times the 50% lethal dose of the infecting organisms. Ten animals were used at each of the serial fourfold dose concentrations of the antibiotics tested. Antibiotics were given subcutaneously (0.5 ml per animal) at 1 and 5 h after challenge. The median effective dose ($ED_{50}$) was calculated from the number of surviving animals on day 7 by the method of Reed and Muench (10).

## RESULTS

The comparison compounds used in this study were chosen to be representative of cephalosporins recently introduced into the clinic (cefotax-

TABLE 1. Stability of azthreonam and comparison compounds to chromosomally mediated β-lactamases isolated from gram-negative bacteria

| Compound | Relative efficiency[a] of hydrolysis by β-lactamase type: | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Klebsiella* K1 | *Proteus* P1 | *Serratia* S1 | *Enterobacter* P99 | *Enterobacter* E1 | *Providencia* PD1 | *Bacteroides* B1 |
| Cephaloridine | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Azthreonam | 10 | <1.0[b] | <0.2[b] | <0.01[b] | <0.01 | 0.03 | 1.1 |
| Ceftazidime | 0.01 | <0.1[b] | <0.2[b] | 0.2 | 0.04 | 1.3 | 4.0 |
| Cefotaxime | 5.0 | 0.6 | 5.0 | 5.0 | 0.02 | 3.0 | 30 |
| Cefoperazone | 30 | >1[c] | 30 | 7.0 | 5.0 | 5.0 | 40 |

[a] Relative efficiency is defined as described in the text.
[b] Relative $V_{max}$ value.
[c] Nonlinear kinetics.

ime and cefoperazone) and those in the final stages of development (ceftazidime).

**β-Lactamase stability.** The stability of azthreonam and comparison compounds to chromosomally mediated β-lactamases isolated from clinically significant gram-negative organisms is shown in Table 1. Cephaloridine is included as a reference compound and designated a relative value of 100 for susceptibility to enzyme hydrolysis. With the exception of the K1-enzyme produced by certain strains of *Klebsiella*, azthreonam shows a high degree of stability to all of the enzymes tested. Susceptibility of azthreonam to hydrolysis by the K1 enzyme was similar to that observed with cefotaxime and less than cefoperazone. β-Lactamase produced by strains of *Enterobacter, Providencia*, and *B. fragilis* hydrolyzed azthreonam less efficiently than did the comparison compounds.

In addition, azthreonam showed a high degree of stability to R-plasmid-mediated lactamases (Table 2). The commonly occurring TEM enzymes showed little effect against azthreonam and the aminothiazoyl oxime cephalosporins (cefotaxime and ceftazidime); in contrast, cefoperazone was highly susceptible to hydrolysis. Similar results were observed with the less commonly occurring OXA-2 and SHV-1 β-lactamases. Among the *Pseudomonas*-specific enzymes (PSE 1-4), PSE-2 showed slight hydrolysis of azthreonam, moderate hydrolysis of cefotaxime, and high activity against cefoperazone. Ceftazidime was highly stable to hydrolysis by these enzymes.

**Antibacterial activity.** The antimicrobial spectrum of azthreonam is unique among β-lactam antibiotics. The compound is marginally active against gram-positive organisms and anaerobes, but has exceptional activity against aerobic gram-negative bacteria, including *P. aeruginosa* (Table 3). With the exception of *Streptococcus pyogenes*, azthreonam showed virtually no activity against the gram-positive bacteria tested. Activity against *S. pyogenes* was at least 2 to 3 orders of magnitude less than that observed with the comparison compounds. All strains of *E. coli, S. marcescens, Proteus* sp., *Salmonella, Providencia, Haemophilus influenzae*, and *Neisseria gonorrhoeae* were highly susceptible to azthreonam, irrespective of their ability to produce β-lactamase. Cefoperazone was the least active compound tested against these organisms, due mainly to its instability to β-lactamases. Azthreonam showed exceptional activity against *Proteus* and *Providencia* sp. Azthreonam also showed a high degree of activity against strains of *K. pneumoniae*, inhibiting 90% of the strains tested at 0.3 μg/ml. However, two strains were resistant due to production of large amounts of the chromosomally mediated K1 enzyme. Strains of *Enterobacter* have consistently defied attack by β-lactam antibiotics, due

TABLE 2. Stability of azthreonam and comparison compounds to plasmid-mediated β-lactamases isolated from gram-negative bacteria

| Compound | Relative efficiency[a] of hydrolysis by β-lactamase type: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | TEM-1 | TEM-2 | OXA-2 | SHV-1 | PSE-1 | PSE-2 | PSE-3 | PSE-4 |
| Cephaloridine | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Azthreonam | 0.04 | 0.03 | 1.0 | 0.1 | 0.02 | 5.5 | 0.8 | 0.01 |
| Ceftazidime | <0.01 | <0.01 | <0.5 | 0.04 | 0.02 | <3.0 | 0.4 | <0.01 |
| Cefotaxime | 0.1 | 0.07 | 6.0 | 0.14 | 0.03 | 40 | 0.3 | 0.03 |
| Cefoperazone | 70 | 60 | 250 | 150 | 260 | 1900 | 6 | 280 |

[a] Relative efficiency is defined as described in the text.

TABLE 3. Antibacterial activity of azthreonam and other antibiotics

| Organism (no. of strains) | Compound | MIC range (μg/ml) | MIC (μg/ml) needed to inhibit: | |
|---|---|---|---|---|
| | | | 50% of strains | 90% of strains |
| *Staphylococcus aureus* (12) | Azthreonam | >100 | >100 | >100 |
| | Cefotaxime | 0.4–12.5 | 1.6 | 3.1 |
| | Ceftazidime | 6.3–50 | 7.3 | 12.5 |
| | Cefoperazone | 0.8–12.5 | 1.3 | 2.8 |
| *Streptococcus pyogenes* (11) | Azthreonam | 12.5–>100 | 9.8 | 12.5 |
| | Cefotaxime | <0.1 | <0.1 | <0.1 |
| | Ceftazidime | 0.1–0.2 | 0.1 | 0.2 |
| | Cefoperazone | <0.1–0.8 | <0.1 | 0.1 |
| *Streptococcus pneumoniae* (11) | Azthreonam | 50 –>100 | >100 | >100 |
| | Cefotaxime | <0.1 | <0.1 | <0.1 |
| | Ceftazidime | <0.1–0.4 | 0.3 | 0.4 |
| | Cefoperazone | <0.1–0.2 | <0.1 | <0.1 |
| *Streptococcus faecalis* (12) | Azthreonam | >100 | >100 | >100 |
| | Cefotaxime | 1.6–>100 | >100 | >100 |
| | Ceftazidime | 50 –>100 | >100 | >100 |
| | Cefoperazone | 6.3–50 | 12.5 | 24.4 |
| *Escherichia coli* (79) | Azthreonam | <0.1–0.8 | 0.1 | 0.2 |
| | Cefotaxime | <0.1–0.8 | <0.1 | 0.2 |
| | Ceftazidime | <0.1–0.8 | 0.1 | 0.4 |
| | Cefoperazone | <0.1–>100 | 0.2 | >100 |
| *Klebsiella pneumoniae* (68) | Azthreonam | <0.1–>100 | <0.1 | 0.3 |
| | Cefotaxime | <0.1–100 | <0.1 | 0.3 |
| | Ceftazidime | <0.1–6.3 | 0.2 | 1.1 |
| | Cefoperazone | <0.1–>100 | 0.6 | >100 |
| *Enterobacter cloacae* (29) | Azthreonam | <0.1–50 | 0.2 | 12.5 |
| | Cefotaxime | 0.1–>100 | 0.5 | 25.0 |
| | Ceftazidime | 0.2–>100 | 0.7 | 12.5 |
| | Cefoperazone | 0.1–>100 | 0.5 | 100 |
| *Enterobacter aerogenes* (13) | Azthreonam | 0.1–50 | 0.1 | 33.3 |
| | Cefotaxime | 0.1–>100 | <0.1 | 42.9 |
| | Ceftazidime | 0.2–>100 | 0.5 | >100 |
| | Cefoperazone | 0.1–>100 | 0.2 | >100 |
| *Serratia marcescens* (113) | Azthreonam | 0.1–6.3 | 0.3 | 1.6 |
| | Cefotaxime | 0.2–50 | 0.5 | 8.4 |
| | Ceftazidime | 0.1–6.3 | 0.4 | 1.5 |
| | Cefoperazone | 1.6–>100 | 5.6 | >100 |
| *Proteus mirabilis* (25) | Azthreonam | <0.1–0.1 | <0.1 | <0.1 |
| | Cefotaxime | <0.1–1.6 | <0.1 | <0.1 |
| | Ceftazidime | <0.1–0.4 | <0.1 | 0.1 |
| | Cefoperazone | 0.8–3.1 | 1.2 | 2.4 |
| *Proteus vulgaris* (11) | Azthreonam | <0.1–0.1 | <0.1 | <0.1 |
| | Cefotaxime | <0.1–100 | <0.1 | 5.5 |
| | Ceftazidime | <0.1–0.2 | <0.1 | <0.1 |
| | Cefoperazone | <0.1–>100 | 1.4 | 5.8 |
| *Proteus rettgeri* (6) | Azthreonam | <0.1 | <0.1 | <0.1 |
| | Cefotaxime | <0.1–0.2 | 0.1 | 0.2 |
| | Ceftazidime | <0.1–3.1 | 0.4 | 2.3 |
| | Cefoperazone | 0.2–12.5 | 3.1 | 10.7 |
| *Proteus morganii* (19) | Azthreonam | <0.1–1.6 | <0.1 | 0.6 |
| | Cefotaxime | <0.1–12.5 | <0.1 | 1.6 |
| | Ceftazidime | <0.1–>100 | 0.2 | 3.1 |
| | Cefoperazone | 0.4–>100 | 2.2 | 6.3 |
| *Providencia stuartii* (15) | Azthreonam | <0.1–0.1 | <0.1 | <0.1 |
| | Cefotaxime | 0.1–6.3 | 1.2 | 3.0 |
| | Ceftazidime | 0.2–6.3 | 1.2 | 4.7 |
| | Cefoperazone | 1.6–>100 | 10.3 | >100 |
| *Shigella* sp. (25) | Azthreonam | <0.1–12.5 | <0.1 | 5.7 |
| | Cefotaxime | <0.1–6.3 | <0.1 | 3.6 |
| | Ceftazidime | 0.1–6.3 | 0.2 | 4.3 |
| | Cefoperazone | <0.1–>100 | 0.2 | 43.8 |

TABLE 3—*Continued*

| Organism (no. of strains) | Compound | MIC range (μg/ml) | MIC (μg/ml) needed to inhibit: | |
|---|---|---|---|---|
| | | | 50% of strains | 90% of strains |
| *Salmonella* sp. (25) | Azthreonam | 0.1–0.8 | 0.1 | 0.3 |
| | Cefotaxime | <0.1–0.4 | 0.2 | 0.3 |
| | Ceftazidime | 0.4–12.5 | 0.6 | 1.4 |
| | Cefoperazone | 0.4–>100 | 0.6 | 75.0 |
| *Citrobacter freundii* (25) | Azthreonam | <0.1–50 | 0.2 | 0.7 |
| | Cefotaxime | <0.1–50 | 0.1 | 0.4 |
| | Ceftazidime | 0.2–>100 | 0.6 | 2.0 |
| | Cefoperazone | 0.1–>100 | 0.6 | 4.7 |
| *Acinetobacter calcoaceticus* (25) | Azthreonam | 1.6–100 | 20.1 | 58.3 |
| | Cefotaxime | 0.2–100 | 12.0 | 24.4 |
| | Ceftazidime | 0.4–25 | 5.2 | 11.4 |
| | Cefoperazone | 12.5–>100 | 93.8 | >100 |
| *Pseudomonas aeruginosa* (61) | Azthreonam | 0.2–50 | 4.3 | 12.0 |
| | Cefotaxime | 0.8–100 | 16.2 | 47.8 |
| | Ceftazidime | 0.8–12.5 | 1.5 | 3.2 |
| | Cefoperazone | 1.6–>100 | 5.6 | 12.0 |
| *Haemophilus influenzae* (18) (ampicillin susceptible) | Azthreonam | <0.1–0.2 | <0.1 | 0.2 |
| | Cefotaxime | <0.1 | <0.1 | <0.1 |
| | Ceftazidime | <0.1–0.4 | <0.1 | 0.2 |
| | Cefoperazone | <0.1–0.2 | <0.1 | 0.1 |
| *Haemophilus influenzae* (18) (ampicillin resistant) | Azthreonam | <0.1–0.2 | <0.1 | 0.2 |
| | Cefotaxime | <0.1 | <0.1 | <0.1 |
| | Ceftazidime | <0.1–0.2 | <0.1 | <0.1 |
| | Cefoperazone | 3.1–>100 | 60.7 | >100 |
| *Neisseria gonorrhoeae* (20) | Azthreonam | <0.1–0.4 | <0.1 | 0.2 |
| | Cefotaxime | <0.1 | <0.1 | <0.1 |
| | Ceftazidime | <0.1 | <0.1 | <0.1 |
| | Cefoperazone | <0.1–0.8 | <0.1 | 0.2 |
| *Bacteroides fragilis* (8) | Azthreonam | 100 –>100 | >100 | >100 |
| | Cefotaxime | 25 –>100 | 100 | >100 |
| | Ceftazidime | 100 –>100 | >100 | >100 |
| | Cefoperazone | >100 | >100 | >100 |

mainly to the production of chromosomally mediated class I β-lactamase (15), combined with a relatively inpenetrable outer membrane (14). Although strains of *E. cloacae* were relatively susceptible to azthreonam, cefotaxime, and ceftazidime, *E. aerogenes* strains exhibited a greater degree of resistance to all the compounds tested. The majority of *Shigella* strains produced large amounts of β-lactamase and a number of strains were relatively resistant to all compounds tested. *Citrobacter* strains were generally susceptible to azthreonam and comparison compounds, although resistant strains were observed. Strains of *Acinetobacter* were relatively resistant to all compounds, with ceftazidime being overall the most active. Strains of *P. aeruginosa* were on the whole susceptible to the compounds tested, with cefotaxime being the least active. None of the compounds showed significant activity against *B. fragilis*.

The effect of inoculum size on the activity of azthreonam against β-lactamase-producing bacteria is shown in Table 4. Little or no inoculum effect was observed with azthreonam against strains of *E. coli, Proteus* sp., *Providencia* sp., *S. marcescens*, and *P. aeruginosa*. A pronounced inoculum effect was observed with all compounds against strains of *Enterobacter* and K1-producing strains of *Klebsiella*. Overall, cefoperazone activity showed the greatest variation with changes in inoculum size, whereas azthreonam and ceftazidime were the least affected. The effect of inoculum size on the activity of azthreonam against R-plasmid-carrying strains of *P. aeruginosa* is shown in Table 5. Azthreonam, cefotaxime, and ceftazidime exhibited relatively small changes in activity with increased inoculum. Cefoperazone and to a lesser extent cefsulodin, showed the largest variations in activity.

**Interaction with penicillin-binding proteins.** Azthreonam has been studied with respect to effects on bacterial morphology and binding to penicillin-binding proteins of a range of gram-negative bacteria. In all organisms examined, azthreonam caused filamentation at its lowest effective concentration. The PBP profiles (Table 6) indicated a very high affinity of azthreonam

TABLE 4. Effect of inoculum size on activity of azthreonam and comparison compounds against β-lactamase-producing bacteria

| Organism (no. tested) | Compound | $MIC_{90}$[a] (μg/ml) at inoculum size (CFU) | | |
|---|---|---|---|---|
| | | $10^2$ | $10^4$ | $10^6$ |
| *E. coli* (20) | Azthreonam | <0.1 | <0.1 | <0.1 |
| | Cefotaxime | <0.1 | <0.1 | <0.1 |
| | Ceftazidime | <0.1 | <0.1 | <0.1 |
| | Cefoperazone | 2.4 | 8.8 | >100 |
| *Klebsiella* sp. (20) | Azthreonam | 3.1 | 16.1 | 50 |
| | Cefotaxime | <0.2 | 0.8 | 25 |
| | Ceftazidime | 0.8 | 1.3 | 9.1 |
| | Cefoperazone | 25 | >100 | >100 |
| *P. morganii* and *P. vulgaris* (20) | Azthreonam | <0.1 | <0.1 | <0.1 |
| | Cefotaxime | <0.1 | <0.1 | >100 |
| | Ceftazidime | <0.1 | 0.5 | 29.2 |
| | Cefoperazone | 3.6 | 7.4 | >100 |
| *Providencia* sp. (19) | Azthreonam | <0.1 | <0.1 | <0.1 |
| | Cefotaxime | <0.1 | <0.1 | 11.0 |
| | Ceftazidime | 0.2 | 0.6 | 2.4 |
| | Cefoperazone | 6.3 | 6.3 | >100 |
| *Enterobacter* sp. (20) | Azthreonam | 19.3 | 42.2 | 68.8 |
| | Cefotaxime | 54.5 | 68.8 | >100 |
| | Ceftazidime | 29.2 | 54.5 | >100 |
| | Cefoperazone | 7.4 | 34.4 | >100 |
| *S. marcescens* (20) | Azthreonam | 1.2 | 1.2 | 1.9 |
| | Cefotaxime | 10.8 | 18.2 | 21.4 |
| | Ceftazidime | 1.2 | 1.2 | 1.4 |
| | Cefoperazone | >100 | >100 | >100 |
| *P. aeruginosa* (20) | Azthreonam | 5.2 | 5.9 | 12.5 |
| | Cefotaxime | 12.5 | 21.4 | 50 |
| | Ceftazidime | 1.4 | 2.4 | 3.1 |
| | Cefoperazone | 3.0 | 3.1 | 11.6 |
| | Cefsulodin | 1.2 | 2.4 | 3.0 |
| *B. fragilis* (15) | Azthreonam | >100 | >100 | >100 |
| | Cefotaxime | 75 | >100 | >100 |
| | Ceftazidime | >100 | >100 | >100 |
| | Cefoperazone | 100 | >100 | >100 |
| | Cefoxitin | 3.0 | 3.1 | 3.1 |

[a] $MIC_{90}$, Concentration required to inhibit 90% of strains.

TABLE 5. Effect of inoculum size on activity of azthreonam and comparison compounds against strains of *P. aeruginosa* producing R-plasmid-mediated β-lactamases

| β-Lactamase | MIC (μg/ml) at inoculum size (CFU): | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Azthreonam | | | Cefotaxime | | | Ceftazidime | | | Cefoperazone | | | Cefsulodin | | |
| | $10^2$ | $10^4$ | $10^6$ | $10^2$ | $10^4$ | $10^6$ | $10^2$ | $10^4$ | $10^6$ | $10^2$ | $10^4$ | $10^6$ | $10^2$ | $10^4$ | $10^6$ |
| TEM-1 | 1.6 | 3.1 | 3.1 | 3.1 | 6.3 | 12.5 | 3.1 | 3.1 | 3.1 | 6.3 | 50 | >100 | 25 | 50 | >100 |
| TEM-2 | 1.6 | 3.1 | 6.3 | 6.3 | 12.5 | 25 | 0.4 | 0.8 | 0.8 | 12.5 | 50 | >100 | 25 | 50 | >100 |
| OXA-2 | 1.6 | 3.1 | 6.3 | 6.3 | 12.5 | 25 | 1.6 | 3.1 | 12.5 | 12.5 | 12.5 | 50 | 1.6 | 3.1 | 6.3 |
| OXA-3 | 1.6 | 3.1 | 6.3 | 12.5 | 12.5 | 25 | 3.1 | 6.3 | 12.5 | 12.5 | 12.5 | 25 | 3.1 | 3.1 | 6.3 |
| SHV-1 | 1.6 | 3.1 | 6.3 | 6.3 | 6.3 | 6.3 | 0.4 | 0.8 | 1.6 | 12.5 | 12.5 | >100 | 0.8 | 0.8 | 3.1 |
| PSE-1 | 0.8 | 1.6 | 6.3 | 3.1 | 6.3 | 25 | 0.8 | 0.8 | 1.6 | 12.5 | 25 | 50 | 12.5 | 12.5 | 50 |
| PSE-2 | 0.8 | 1.6 | 6.3 | 3.1 | 6.3 | 25 | 0.4 | 0.4 | 0.8 | 12.5 | 12.5 | >100 | 0.8 | 3.1 | 12.5 |
| PSE-3 | 0.8 | 1.6 | 6.3 | 3.1 | 6.3 | 25 | 0.4 | 0.8 | 1.6 | 6.3 | 6.3 | 25 | 6.3 | 6.3 | 25 |
| PSE-4 | 0.8 | 1.6 | 6.3 | 3.1 | 6.3 | 50 | 0.4 | 0.8 | 1.6 | 12.5 | 25 | 100 | 6.3 | 12.5 | 25 |

TABLE 6. Inhibition by azthreonam of penicillin G binding to penicillin-binding proteins of gram-negative bacteria

| Organism | μg/ml to completely inhibit penicillin G binding to: | | | | | | MIC[a] (μg/ml) |
|---|---|---|---|---|---|---|---|
| | PBP1a | PBP1b | PBP2 | PBP3 | PBP4 | PBP5/6 | |
| *E. coli* SC 8294 | 10 | ≥100 | >100 | 0.1 | 100 | 100 | 0.1 |
| *P. vulgaris* SC 9416 | 10 | >100 | ND[b] | 0.1 | ND | >100 | <0.05 |
| *E. cloacae* SC 8236 | 10 | >100 | >100 | 0.1 | >100 | 0.1 | 0.1 |
| *K. pneumoniae* SC 9527 | ND | >100 | >100 | 0.1 | >100 | >100 | 0.1 |
| *P. aeruginosa* SC 9545 | ND | 100 | >100 | 0.1 | >100 | >100 | 0.2 |

[a] Determined at an inoculum of $10^5$ CFU.
[b] ND, Not determined.

for PBP3 (complete binding at 0.1 μg/ml), moderate affinity for PBP1a and poor affinity for PBP1b, -2, -4 and -5/6.

**Efficacy in systemic murine infections.** The results of protection tests in mice challenged with gram-negative bacteria are given in Table 7. In general, the efficacies of azthreonam, cefotaxime, and ceftazidime were similar, although azthreonam was superior to the comparison compounds against *P. stuartii* both in terms of MIC and $ED_{50}$ values. Cefoperazone was generally less effective than the other compounds tested, especially against organisms producing β-lactamase.

## DISCUSSION

Azthreonam, a novel monocyclic β-lactam antibacterial agent, exhibits a unique pattern of antibacterial activity when compared to previously studied penicillins and cephalosporins. The compound is highly active against aerobic gram-negative bacteria, including *P. aeruginosa*, while exhibiting little or no activity against gram-positive bacteria and anaerobes. The exceptional activity of azthreonam appears to be a function of its high and somewhat exclusive affinity for essential penicillin-binding protein 3 of gram-negative bacteria and stability to a wide range of β-lactamases produced by these clinically important pathogens. The spectrum of activity encompasses all members of the *Enterobacteriaceae*, *P. aeruginosa*, *H. influenzae*, and *N. gonorrhoeae*. Azthreonam possesses a high degree of resistance to enzymatic hydrolysis by most of the common chromosomal and plasmid-mediated β-lactamases studied. The only enzyme showing any appreciable destruction of azthreonam was the relatively uncommon K1 β-lactamase produced by some strains of *K. pneumoniae* (15). In mouse protection tests the good performance of azthreonam was in keeping with its in vitro activity.

Azthreonam is an antimicrobial agent specifically targeted against multiply resistant gram-negative bacteria which are commonly and increasingly being implicated in nosocomial infections. The lack of activity against gram-positive bacteria and anaerobes may well be advantageous for such a compound by reducing the potential for gastrointestinal disturbances and leaving the normal gram-positive flora of the body undisturbed. We are encouraged by our findings so far, and azthreonam is presently being progressed into the clinic.

TABLE 7. Efficacy of azthreonam in systemic murine infections

| Pathogen | Azthreonam | | Cefotaxime | | Ceftazidime | | Cefoperazone | |
|---|---|---|---|---|---|---|---|---|
| | MIC[a] (μg/ml) | $ED_{50}$ (mg/kg) | MIC (μg/ml) | $ED_{50}$ (mg/kg) | MIC (μg/ml) | $ED_{50}$ (mg/kg) | MIC (μg/ml) | $ED_{50}$ (mg/kg) |
| *E. coli* SC 8294 | 0.4 | 0.4 | 0.1 | 0.2 | 0.4 | <0.1 | 0.8 | 0.2 |
| *S. sonnei* SC 8449 | 0.2 | 0.3 | <0.1 | 0.08 | 0.2 | 0.2 | 0.4 | 0.5 |
| *S. schottmulleri* SC 3850 | <0.1 | 0.2 | <0.1 | 0.2 | 0.1 | 0.2 | <0.1 | 0.7 |
| *K. pneumoniae* SC 12216 | 0.1 | 0.4 | <0.1 | 0.2 | 0.4 | 0.3 | 3.1 | 7.9 |
| *C. freundii* SC 10204 | 0.1 | 0.9 | 0.1 | 3.1 | 0.4 | 0.5 | 0.2 | 1.0 |
| *E. cloacae* SC 9965 | 0.1 | 3.7 | 0.1 | 24.4 | 0.2 | 3.6 | 0.4 | 5.9 |
| *P. rettgeri* SC 8217 | <0.1 | 0.6 | <0.1 | 0.3 | <0.1 | 0.7 | 1.6 | 65.2 |
| *P. stuartii* SC 12375 | <0.1 | 0.4 | 1.6 | 7.0 | 3.1 | 18.8 | 12.5 | >100 |
| *H. influenzae* SC 10556 | <0.1 | 0.4 | <0.1 | <0.1 | <0.1 | 0.1 | 50 | 0.1 |
| *S. marcescens* SC 9782 | 0.2 | 0.5 | 1.6 | 0.6 | 0.2 | 0.7 | 1.6 | 28.5 |
| *P. aeruginosa* SC 8392 | 3.1 | 24.7 | 12.5 | 48.0 | 1.6 | 37.0 | 3.1 | >200 |

[a] Determined at an inoculum of $10^5$ CFU.

## ACKNOWLEDGMENTS

We extend our gratitude to Joan Freudenberger, Steven Olsen, Rosalie Schwind, Sandra Smith, and Rita Whitney for their expert technical assistance.

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